REMARKS

With this Amendment, Claims 1-8, 12 and 16 have been amended and Claims 17-29 have been canceled without prejudice. Claim 31 has been added. After entry of this Amendment, Claims 1-16, 30 and 31 are pending in the instant Application. Applicants expressly reserve the right to prosecute Claims drawn to canceled subject matter in one or more continuation, divisional or continuation-in-part applications.

AMENDMENT OF CLAIMS

Claim 1 has been amended to clarify the claimed invention. Support for the amendment of Claim 1 may be found in the Specification at page 3, lines 24-26. Claims 2-8 have been amended to clarify the claimed invention. Support for the amendment of Claims 2-8 may be found in the Specification at page 10, lines 10-14. Claim 12 has been amended to recite that the assay is an *in vivo* assay and is supported by the Specification at page 10, line 3. Claim 16 has been amended to recite that the peptide comprises a nuclear receptor box (NR-box) amino acid sequence or derivative thereof and is supported by Figures 6, 7 and 10.

New Claim 31 is supported by originally filed Claim 17.

No new matter is added by the amendment of Claims 1-8, 12 and 16 and the addition of new Claim 31. Accordingly, entry into the instant Application is proper and respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 112, first paragraph

Claim 16 stands rejected under 35 U.S.C. § 112, first paragraph for containing new matter. More particularly, the Patent Office asserted that the term "NR" was not defined anywhere in the Specification. Applicants respectfully traverse the rejection.

The specification need not provide written description support in exactly the same words as are used in the claims. *Application of Luckach* 169 USPQ 795, 796 (CCPA 1971). It is enough that the specification conveys to those of skill in the art that the applicant had possession of the invention. *In re Wilder* 222 USPQ 369, 372 (Fed. Cir. 1984).

Applicants submit that the term "NR" is well known to those of skill in the art to as an abbreviation of "nuclear receptor." Applicants refer the Patent Office to Appendix A (*i.e.*, Geistlinger *et al., J. Am. Chem. Soc.* **2001**, 123 1525-1526) where NR is defined in the second

line of page 1525. Accordingly Applicants submit that the term "NR box" is well known to those of skill in the art as an abbreviation of "nuclear receptor box."

Applicants point out that the term "NR box" is used extensively in the prior art cited in this application. Further, Applicant refer the Patent Office to Appendix A (*i.e.*, Geistlinger *et al.*, *J. Am. Chem. Soc.***2001**, 123 1525-1526) where NR box is defined in the second paragraph of page 1525. Accordingly, since the term "NR box" is well-known to those of skill in the art a definition of "NR box" need not be taught in the instant Specification. Further, Applicants have provided examples of an "NR box" in Figures 6, 7, 10 and 11. Accordingly, the skilled artisan would immediately understand that the applicant had possession of the invention recited in Claim 16, as amended.

Applicants point out that Claim 16, as amended, does not recite "nuclear receptor box motif," which obviate the new matter rejection on this basis.

In view of the foregoing, Applicants respectfully request that the rejection of Claim 16 under 35 U.S.C. § 112, first paragraph be withdrawn.

Claims 1-17 and 30 stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. More specifically, the Patent Office maintained that, while the specification was enabling for methods for identifying compounds which modulate coactivator binding to nuclear receptors with coactivator binding sites, the specification failed to provide enablement for a similar method where the nuclear receptors lack a coactivator binding site. Applicants respectfully traverse the rejection.

In order to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph a patent application, supplemented with information known in the art, need only teach one of skill in the art how to make and use the invention, without undue experimentation. Accordingly, the enablement requirement is fulfilled when the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim. *In re Fisher* 166 USPQ 18, 24 (CCPA 1970). The patent disclosure is not required to teach, and preferably omits that which is well known in the art. *In re Buchner* 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 231 USPQ 81, 94 (Fed. Cir. 1986); *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.* 221 USPQ 481, 489 (Fed. Cir. 1984). Experimentation typically engaged in by those of skill in the art is permitted, as long as the experimentation is

not undue. *In re Wands* 8 USPQ2d 1400, 1404 (Fed. Cir. 1988); *In re Angstadt* 190 USPQ 214, 219 (CCPA 1976).

A disclosure, as filed, is presumed to be enabled, unless there is reason to objectively doubt the truth of the statements relied on for enabling support. *In re Marzocchi* 169 USPQ 367, 370 (CCPA 1971). Thus, the Patent Office bears the initial burden of presenting a reasonable explanation of why the scope of protection sought in the claims is not enabled by the specification. *In re Wright* 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Accordingly, the Patent Office must, at minimum, provide specific technical reasons, which explain how the specification fails to enable the claimed invention. *In re Bowen* 181 USPQ 48, 51 (CCPA 1974); MPEP § 2164.04.

Independent Claim 1, as amended, recites a method of identifying a compound that binds to a coactivator binding site of a nuclear receptor. Applicants submit that Claim 1 is fully enabled by the Specification, as filed.

The Specification teaches identification (Specification at page 9, line 30 to page 10, line 23) and modeling (Specification at page 10, line 23 to page 11, line 4) of coactivator binding sites of a nuclear receptor by homology to the coactivator binding site of human thyroid receptor. Further the Specification teaches numerous methods for modeling whether a test compound fits spatially into a nuclear coactivator binding site (Specification, page 11, line 5 to page 11, line 34 and page 13, line 3 to page line 35). Finally the Specification teaches methods for screening the test compounds in assays which measure the binding of a test compound to the nuclear activator binding site (page 15, line to page 15, line 31), which consequently allow for identification of a test compound which binds to the nuclear receptor coactivator binding site.

Thus, the Specification discloses detailed procedures for making and using the claimed invention that bears a reasonable correlation to the scope of the claimed invention. Further, in view of the extensive guidance provided by the Specification, Applicants respectfully submit that the amount of experimentation required to practice the claimed invention is not undue. Thus, Applicants maintain that the claimed invention may be practiced with any nuclear receptor which has a coactivator binding site, regardless of whether the nuclear receptor is "known" to have a coactivator binding site (*i.e.*, regardless of whether the receptor has a known ligand which binds to the coactivator binding site) without undue experimentation,

given the detailed disclosure of the Specification. Accordingly, the Specification teaches how to make and use the claimed invention without undue experimentation. Nothing more is required by 35 U.S.C. § 112, first paragraph.

Applicants further point out that every possible embodiment of a generic claim need not be disclosed in the specification to satisfy the requirements of 35 U.S.C. § 112, first paragraph. *Genentech, Inc. v. Novo Nordisk* 42 USPQ2d 1001 (Fed. Cir. 1997). Accordingly, Applicants are entitled to a generic claim without providing specific guidance or working examples of every possible embodiment of the claimed invention.

In view of the foregoing, Applicants respectfully request that the rejection of Claims 1-17 and 30 under 35 U.S.C. § 112, first paragraph be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 112, second paragraph

Claims 1-17 and 30 stand rejected under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. Applicants respectfully traverse the rejection.

The Patent Office asserted that Claim 1 failed to recite a step that measured coactivator binding to a nuclear coactivator binding site. Claim 1, as amended, recites "screening said test compounds in an assay that measures binding of a test compound to a nuclear coactivator binding site." Accordingly, Claim 1, as amended, recites measurement of the binding of a test compound to a nuclear coactivator binding site, which obviates the rejection.

The Patent Office asserted that Claim 17 failed to recite a step that measured coactivator binding to a nuclear coactivator binding site. Claim 17 has been canceled, which obviates the rejection.

Claim 12 has been amended to recite that the assay is an *in vivo* assay, which obviates the rejection.

Applicants submit that the amendment of Claims 2-8 where "corresponding to" been replaced by "identified by homology alignment" obviates the rejection.

Claim definiteness is determined under 35 U.S.C. § 112, second paragraph by analyzing whether the skilled artisan could understand the bounds of the claim when read in view of the specification. *Credle v. Bond* 30 USPQ2d 1911 (Fed. Cir. 1994). Accordingly, if those of skill in the art are reasonably apprised of the scope of a claim, then the claim can be considered definite. *In re Waterdam* 31 USPQ2d 1754 (Fed. Cir. 1994).

The Patent Office claimed that the term "nuclear receptor box" was indefinite because "NR box" was not specifically defined in the Specification or Claims. Applicants have already pointed out that explicit definition of a term well-known to the skilled artisan is not required. Accordingly, the skilled artisan would immediately comprehend the metes and bounds of a claim which recited nuclear receptor boxes, which obviates the rejection.

Applicants further submit that the subsequent comments by the Patent Office are misplaced. Numerous nuclear receptor boxes for nuclear receptors are known in the art (see Figure 6, paragraph 2, page 1525 of Geistlinger *et al.*, *J. Am. Chem. Soc.***2001**, 123 1525-1526 provided in Appendix A). Accordingly, Applicants are not limited to the nuclear receptor boxes described in the instant Specification and Claims.

Nevertheless, Applicants, address the subsequent allegations in attempt to dispel any confusion on the part of the Patent Office. Applicants point out that NR-box 1 in Figure 7 is residues 15-21 of SEQ ID NO: 5. Figure 7 erroneously represents that NR-box 1 in Figure 7 may also be residues 15-21 of SEQ ID NO: 6. In fact, NR-box 2 is residues 15-21 of SEQ ID NO: 6 (please compare SEQ ID 6 in Figure 6 with residues 15-21 of SEQ ID NO: 6 in Figure 7).

Applicants further point out that NR-boxes are part of a nuclear receptor (*i.e.*, part of a large polypeptide) and are not peptides themselves. However, synthetic peptides derived from various NR-boxes may be made synthetically. Accordingly, for example, a peptide derived from NR-box 1 may comprise all or a portion of residues 15-21 of Seq ID NO 5. Similarly, a peptide derived from NR-box 2 may comprise all or a portion of residues 15-21 of Seq ID NO 6 and a peptide derived from NR-box 3 may comprise all or a portion of residues 15-21 of Seq ID NO 7.

In view of the foregoing, Applicants respectfully request that the rejection of Claims 1-16 and 30 under 35 U.S.C. § 112, second paragraph be withdrawn.

REJECTION UNDER 35 U.S.C. § 102 (b)

Claims 1, 10, 12-15, 17 and 30 stand rejected under 35 U.S.C. § 102 (b) as being anticipated by Scanlan *et al.*, International Application No. WO 97/221993 (hereinafter "Scanlan"). Applicants traverse the rejection.

Anticipation of a claim requires that the reference teach every element of the claim. MPEP § 2131. Thus, "a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California* 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

The claimed invention is a method of identifying a compound that binds to a coactivator binding site of a nuclear receptor. First, test compounds that fit spatially into the nuclear receptor coactivator binding site are modeled using an atomic structural model of the nuclear receptor coactivator binding site or portion thereof. Second, the test compounds are screened in an assay that measures binding of a test compound to the nuclear receptor coactivator binding site. Third, a test compound that binds to the coactivator binding site of the nuclear receptor is identified.

Contrastingly, Scanlan teaches a method for identifying compounds which bind to the ligand binding site of a nuclear receptor. Scanlan fails to teach or suggest a method for identifying compounds which bind to a coactivator binding site of a nuclear receptor. Accordingly, since Scanlan fails to teach each and every element of the invention recited in Claim 1, as amended, the cited reference fails to anticipate amended Claim 1.

Claims 10, 12-15 and 30 depend either, directly or indirectly, from independent Claim 1 and are thus patentable for at least the same reason. In view of the foregoing, Applicants respectfully request that the rejection of Claims 1, 10, 12-15 and 30 under 35 U.S.C. § 102 (b) over Scanlan be withdrawn.

REJECTIONS UNDER 35 U.S.C. § 103 (a)

Claims 1-10, 12, 14, 15, 17 and 30 stand rejected under 35 U.S.C. § 103 (a) as being unpatentable over Scanlan in view of Collingwood *et al.*, *Proc. Natl. Acad. of Sci.* 94, 248, 1997 (hereinafter "Collingwood"). Applicants respectfully traverse the rejection.

Applicants have shown above that Scanlan does not teach or suggest every limitation of the invention recited in amended Claim 1. Collingwood teaches mutation of Lue 454 in the coactivator binding site to valine diminishes or abolishes the binding of a coactivor to the thyroid hormone β receptor. Collingwood fails to teach or suggest a method for identifying a compound that binds to the coactivator binding site of a nuclear receptor. Accordingly,

Collingwood fails to cure the deficiency of Scanlan to provide the invention recited in Claim 1, as amended.

Claims 2-10, 12, 14, 15 and 30 depend either directly or indirectly from independent Claim 1 and are thus patentable for at least the same reasons as Claim 1. In view of the foregoing, Applicants respectfully request that the rejection of Claims 1-10, 12, 14, 15, and 30 under 35 U.S.C. § 103 (a) over Scanlan in view of Collingwood be withdrawn.

Claims 1, 10, 11-13, 17 and 30 stand rejected under 35 U.S.C. § 103 (a) as being unpatentable over Scanlan in view of Kuntz *et al.*, *Science* 257, 1078, 1992 (hereinafter "Kuntz"). Applicants respectfully traverse the rejection.

Applicants have shown above that Scanlan does not teach or suggest every limitation of the invention recited in amended Claim 1. Kuntz discloses computational strategies for drug design. Kuntz fails to teach or suggest a method for identifying a compound that binds to the coactivator binding site of a nuclear receptor. Accordingly, Kuntz fails to cure the deficiency of Scanlan to provide the invention recited in Claim 1, as amended.

Claims 10, 11-13 and 30 depend either directly or indirectly from independent Claim 1 and are thus patentable for at least the same reasons as Claim 1. In view of the foregoing, Applicants respectfully request that the rejection of Claims 1, 10, 11-13, and 30 under 35 U.S.C. § 103 (a) over Scanlan in view of Kuntz be withdrawn.

CONCLUSION

Applicants respectfully submit that all pending Claims of the captioned Application satisfy all requirements for patentability and are in condition for allowance. An early indication of the same is therefore respectfully requested.

No other fees are believed due in connection with this Amendment. However, the Commissioner is authorized to charge any required fee not included with this Amendment or credit any overpayment to Pennie & Edmonds LLP Deposit Account No. 16-1150 (9811-008-999). A duplicate copy of this sheet is enclosed for such purpose.

If the Examiner determines that prosecution of the instant application would benefit from a telephone interview, the Examiner is invited to call the undersigned attorney at (212) 790-6578.

Respectfully submitted,

Date April 25, 2002

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Enclosure

APPENDIX A

An Inhibitor of the Interaction of Thyroid Hormone Receptor β and Glucocorticoid Interacting Protein 1

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Received August 25, 2000

The thyroid hormone receptors (TR's), members of the nuclear receptor (NR) superfamily,[1] are crucial for normal development and for regulation of metabolism in the adult.[2] The TR's integrate multiple input signals including tissue and developmental context and hormonal levels to produce distinct patterns of gene expression.[3] The TR's are attractive therapeutic targets in cardiovascular disease.[4] Herein we report a novel approach to chemical antagonism of thyroid hormone signaling.

The TR's exert their effects by dynamic regulation of the formation of multiprotein complexes that include coactivators, co-repressors, and the basal transcription machinery.[5] The TR conformation adopted upon binding of thyroid hormone (T_3) allows the binding of coactivating proteins, while that adopted by the unliganded receptor prevents coactivator binding and allows for corepressor binding.[5b,6] Genetic analysis of NR coactivator proteins revealed a highly conserved motif (LXXLL), termed the NR box, that mediates the interaction of NR's and coactivators.[6] Coactivators often have multiple NR boxes of differing affinity that can exhibit either cooperative[7,8] or noncooperative[9a] binding. X-ray crystallographic studies of the complex of T_3, the human thyroid receptor β isoform (hTRβ), and the coactivator Glucocorticoid Receptor Interacting Protein 1 (GRIP1) (T_3·hTRβ-GRIP1) revealed that the NR box binds to the receptor as an amphipathic α-helix.[9] Biochemical studies of T_3·hTRβ-GRIP1 complex formation revealed that the second NR box of GRIP1 had the highest affinity for T_3·hTRβ, suggested that binding involved an induced fit of the NR box helix, and indicated that there was no apparent cooperativity in NR box binding.[9a,10,11] We demonstrate below that α-helical peptidomimetics of the second NR box of GRIP1 strongly inhibit T_3·hTRβ-GRIP1 complex formation.

In previous studies, macrolactam constrained peptides have been used to inhibit protein-protein interactions.[12] In this study, a series of macrolactam constrained GRIP 1 NR box peptides (Table 1) were synthesized by solid-phase peptide synthesis by using the Fmoc strategy, with orthogonal protection of the relevant lactam precursor side chains, followed by on-resin formation of the macrolactam.[13] The compounds synthesized included variation in the location, length, and orientation of the lactam ring.

Table 1. Sequence and Observed Ellipticities of Cyclic Peptides

	Compound	$[\Theta]_{222}/[\Theta]_{max}$	$[\Theta]_{222}/[\Theta]_{208}$
	Ideal Helix	2.63	1.09
1	NH2-^{685}EKHKILHRLLQDS^{697}-COOH	-1.91	0.47
2	(Oregon Green 488)-EKHKILHRLLQDS-COOH	NA	NA
3	Ac-EHHKIKLRLQLDS-COOH	-4.09	0.37
4	c(D^{688}-K^{692})Ac-EKHKILHRLLDKLLQDS-COOH	-0.53	0.71
5	c(K^{688}-D^{692})Ac-EKHKILHRLLDDSLQDS-COOH	-1.81	0.47
6	c(D^{688}-K^{692})Ac-EKHKILHRLLDKLLQDS-COOH	0.20	0.47
7	c(D^{688}-K^{692})Ac-EKHKILHRLLHKLLQDS-COOH	-0.53	0.71
8	c(K^{688}-D^{692})Ac-EKHKILHRLLHDLLQDS-COOH	-0.20	0.81
9	c(E^{689}-K^{692})Ac-EKHKILERLLKDS-COOH	1.41	0.70

The relative numbering scheme for GRIP1 amino acid residues is indicated on peptide 1. The notation c(X−Y) implies formation of a macrolactam between the side chains at the indicated positions. The NR box leucines are underlined. Linear probe and control peptides are above the bold line; constrained peptides are below the bold line.

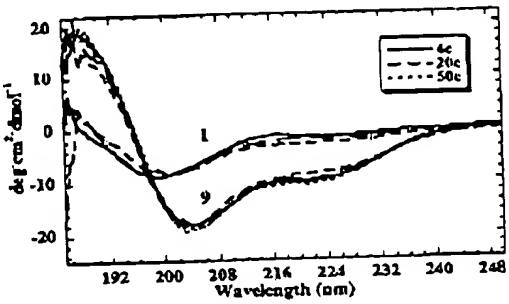

Figure 1. Temperature dependence of circular dichroism spectra of the unconstrained peptide 1 and constrained peptide 9. Spectra were acquired by scanning solutions of 1 and 9 (50 μM in 20% ACN in 50 mM Tris, pH 8.0) at various temperatures. Mean residue ellipticity (Θ) reported in deg·cm^2·dmol^{-1}.

Solution-phase circular dichroism (CD) analysis of these peptides revealed partial induction of α-helical character in one, **9**, but little to no induction of helical character in the other compounds (Table 1). NOESY NMR experiments with **9** revealed four amide proton resonances that show amide-amide (i, $i+1$) cross-peaks characteristic for peptide amides in an α-helical fold with COSY coupling constants less than 5.0 Hz ($J_{HN-H\alpha}$), well within the range normally exhibited in α-helical segments of proteins. These data imply either that peptide **9** has a helical conformation for 30−40% of its length or that populations of energetically similar conformations are interconverting rapidly. The structures of the CD spectra of unconstrained peptide **1** and constrained peptide **9** were independent of the temperature over the range of 4−50 °C (Figure 1) supporting the former model. The combination of these findings suggests that the constraint in **9** strongly biases conformational equilibria toward an α-helical structure within the portion of the peptide enveloped by the lactam.

The ability of the constrained peptides to displace native GRIP1 peptide from T_3·hTRβ was assessed by using fluorescence polarization (FP) equilibrium competition assays (Figure 2). Control experiments indicated that binding of probe **2** was

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10.1021/ja005549c CCC: $20.00
Published on Web 01/30/2001

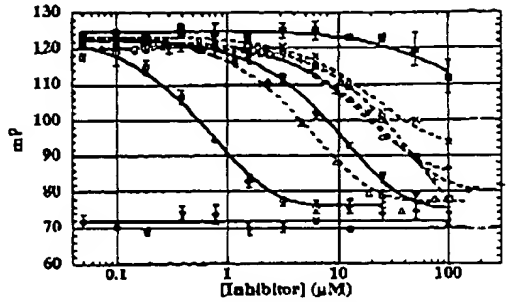

Figure 2. Inhibition of GRIP1 binding to hTRβ by constrained GRIP1 Analogues as determined by fluorescence polarization competition experiments using an Oregon Green 488 labeled GRIP1 peptide 2; (◆) no T3-probe binding is dependent upon the presence of T_3 ligand; (▼) unconstrained GRIP1 peptide 1 specifically blocks binding of 2, IC_{50} of 9.6 ± 0.9 μM; (■) control peptide 3 is unable to block binding of 2; constrained peptides block binding of 2: (+) 4, IC_{50} of 21.8 ± 4.0 μM; (△) 5, IC_{50} of 5.8 ± 0.7 μM; (×) 6, IC_{50} of 22.1 ± 2.2 μM; (◇) 7, IC_{50} of 9.1 ± 2.2 μM; (□) 8, IC_{50} of 20.4 ± 8.6 μM; (○) 9, IC_{50} of 0.62 ± 0.09 μM. [2] was constant at 10 nM, [hTRβ] 1 μM, [T3] 10 μM. Binding buffer: 20 mM Tris HCl at pH 8.0, 100 mM NaCl, 10% glycerol, 1 mM DTT, 0.01% NP-40, and 1 mM EDTA.

dependent upon the presence of T_3 (◆) and that competition for binding to hTRβ was exhibited by unconstrained peptide **1** (▼) but not by control peptide **3**, which has a sequence scrambled NR box (■). While all of the constrained GRIP 1 analogues could successfully compete for binding to hTRβ most did so with affinities worse than or no better than the unconstrained peptide. There were no apparent trends relating the location, length, or orientation of the lactam moiety to relative degree of competitive ability. Strikingly, **9** (○), the single constrained peptide that exhibited substantial helical character, exhibited an IC_{50} 15-fold lower than the unconstrained peptide **1** (▼). The change in inhibitory constant exhibited by **9** indicates that formation of the helix during binding induces a 1.5 kcal/mol cost in free energy of binding. The magnitude of this effect is equivalent with that seen in studies of the yeast transcription factor GCN4 when similar constraints were applied.[14] This study demonstrates that constraint of the NR box to an α-helical structure strongly enhances the affinity of the NR box for the receptor.

The ability of constrained peptide **9** to compete with intact GRIP1 nuclear receptor interaction domain (GRIP1 NID), which contains all three GRIP1 NR boxes, was tested using a semi-quantitative glutathione-*S*-transferase assay. Control experiments indicated that the GRIP1 NID bound to hTRβ in the presence of T_3 (lane 3) and failed to bind in the absence of T_3 (lane 2). This interaction was blocked by unconstrained peptide **1** at high concentration (lane 5) but not at low concentration (lane 4) and was not blocked by control peptide **3** (lane 6). Constrained peptide **9** efficiently blocked the binding of hTRβ to the GRIP1 NID in a dose-dependent manner (lanes 7−10). Although the assay does not allow for exact determination of IC_{50} values, the relative

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Lane	1	2	3	4	5	6		7	8	9	10
^{35}S-hTRβ	+	+	+	+	+	+		+	+	+	+
GST-GRIP1		+	+	+	+	+		+	+	+	+
T3			+	+	+	+		+	+	+	+
1 (μM)				0.01	100						
3 (μM)						100					
9 (μM)								0.01	0.1	1.0	10.0

^{35}S hTRβ ➡

Figure 3. Inhibition of GRIP1 NID protein binding to hTRβ by **9**. Lanes: (1) ^{35}S-hTRβ alone; (2) no T3 hormone, ^{35}S-hTRβ binding is ligand dependent; (3) no competitor maximal binding of ^{35}S-hTRβ to GRIP 1 NID domain; (4) 0.01 μM 1; (5) 100 μM peptide **1** will compete for binding to hTRβ; (6) 100μM 3 showed no competition for binding to hTRβ; (7−10) increasing concentration of **9** conformational constraint increases competitive ability for binding to ^{35}S-hTRβ. ^{35}S-hTRβ was in vitro expressed with ^{35}*S*-methionine. Recombinant fusion of glutathione-*S*-transferase and hGRIP1 (GST-GRIP1$_{(563-767-H6)}$) bound to glutathione agarose beads was exposed to ^{35}S-met-hTRβ in the presence and absence of inhibitor. Binding buffer was 20 mM Tris HCl at pH 8.0, 100 mM NaCl, 10% glycerol, 1 mM DTT, 0.01% NP-40, and 1 mM EDTA.

potency of **9** to **1** was qualitatively in the same range as that observed in the FP studies. Additionally, the interaction was completely blocked by 10 μM **9** whereas the unconstrained peptide never reached this level of saturation, even with 100 μM concentrations. This study indicates that the constrained peptide **9** can block the interaction of intact receptor and coactivator proteins.

Our results suggest that the formation of a Glu-to-Lys macrolactam *c*(E^{691}-K^{695}) in the second NR box of GRIP1 induces a partial α-helical conformation. This conformational constraint allows **9** to compete for the NR box binding site of hTRβ with a 15-fold decrease in IC_{50} relative to GRIP1 thus demonstrating that preforming an α-helical conformation in the NR box leads to stronger interaction between the coactivator and the receptor. The poor competitive abilities of other constrained NR box peptides are most likely due to the lack of α-helicity as indicated by solution CD, thus confirming the requirement for an α-helical conformation in the NR box during binding to the nuclear receptor. The ability to block formation of the T_3·hTRβ·GRIP1 complex should allow functional antagonism of thyroid hormone signaling. Thus, the results of this study suggest that this interface is an appropriate target for the development of protein−protein inhibitors as novel thyroid hormone receptor antagonists.

Acknowledgment. The authors thank R. Flenerick and K. Yamamoto for informative discussions, B. Darimont for technical assistance, V. Doetsch for acquisition of the NMR data, and the Sidney Kimmel Foundation for Cancer Research, the HHMI Research Resources Program grant No. 76296-549901, and the Academic Senate of the University of California at San Francisco for financial support. This paper is dedicated to Joseph L. Goldstein on the occasion of his 60th birthday

Supporting Information Available: Descriptions and results of the synthesis and characterization of the of peptides 1−9, preparation of the allyl protected side chains, circular dichroism, fluorescence polarization, GST pull-down competitions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA005549C

APPENDIX B

MARKED UP VERSION OF AMENDED CLAIMS OF SERIAL NO. 09/281,717

1. (Amended two times) A method of identifying a compound that [modulates] binds to a coactivator binding site of [to] a nuclear receptor, said method comprising:

modeling test compounds that fit spatially into [a] the nuclear receptor coactivator binding site [of interest] using an atomic structural model of [a] the nuclear receptor coactivator binding site or portion thereof,

screening said test compounds in an assay [characterized by] that measures binding of a test compound to [a] the nuclear receptor coactivator binding site, and

identifying a test compound that [modulates] binds to the coactivator binding site [to] of said nuclear receptor.

2. (Amended once) The method of claim 1, wherein said atomic structural model comprises atomic coordinates of amino acid residues [corresponding to] identified by homology alignment with residues of human thyroid receptor selected from the group consisting of Val284, Phe293, Ile302, Leu305, and Leu454.

3. (Amended once) The method of claim 1, wherein said atomic structural model comprises atomic coordinates of amino acid residues [corresponding to] identified by homology alignment with residues of human thyroid receptor selected from the group consisting of Val284, Lys288, Ile302, Lys306, Leu454 and G1u457.

4. (Amended once) The method of claim 1, wherein said atomic structural model comprises atomic coordinates of amino acid residues [corresponding to] identified by homology alignment with residues of human thyroid receptor helix 3 residues I1e280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln30l, Ile302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, G1u457, Val458 and Phe459.

5. (Amended once) The method of claim 1, wherein said nuclear receptor coactivator binding site comprises amino acid residues [corresponding to] identified by

homology alignment with residues of human thyroid receptor selected from the group consisting of helix 3 residues I1e280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln30l, I1e302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, Glu457, Val458 and Phe459.

6. (Amended once) The method of claim 5, wherein said amino acid residues [corresponding to] identified by homology alignment with residues of human thyroid receptor comprise Val284, Phe293, I1e302, Leu305, and Leu454.

7. (Amended once) The method of claim 5, wherein said amino acid residues [corresponding to] identified by homology alignment with residues of human thyroid receptor comprise Val284, Lys288, I1e302, Lys306, Leu454 and G1u457.

8. (Amended once) The method of claim 1, wherein said nuclear receptor coactivator binding site comprises amino acid residues [corresponding to] identified by homology alignment with residues of human thyroid receptor of helix 3 residues Ile280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln30l, Ile302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, G1u457, Val458 and Phe459.

12. (Amended once) The method of claim 1, wherein said assay is a [biological] *in vivo* assay.

16. (Amended two times) The method of Claim 15, wherein the test compound is a peptide comprising a nuclear receptor box [motif] (NR-box) amino acid sequence or derivative thereof.